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# Mitogenic Response of Murine B Lymphocytes to *Salmonella typhimurium* Lipopolysaccharide Requires Protein Kinase C-Dependent Late Tyrosine Phosphorylations

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**Unlike the cross-linking of membrane immunoglobulins, the activation of B cells by lipopolysaccharide (LPS) does not involve the phosphoinositol turnover and the initial activation of tyrosine kinases. However, LPS-induced B-cell proliferation was inhibited by the tyrosine kinase inhibitors genistein and herbimycin A even when added 48 h after the beginning of the culture. Tyrosyl-phosphorylated proteins were detected by Western blotting after 24 h of culture with LPS, reaching a maximum concentration after 72 h. Late tyrosine phosphorylations were also detected in B cells activated for 72 h with anti-immunoglobulin M antibody and were abrogated by the protein synthesis inhibitor cycloheximide, the tyrosine kinase inhibitors genistein and herbimycin A, and the protein kinase C inhibitor chelerythrine. The role of protein kinase C in late tyrosine kinase activation is independent of $Ca^{2+}$ mobilization and was confirmed by detection of a comparable but restricted pattern of tyrosine-phosphorylated substrates in B cells treated with phorbol myristate acetate alone or in association with ionomycin. Tyrosine kinase activation was dependent on de novo protein synthesis. However, culture supernatants of LPS-activated B cells were devoid of mitogenic activity and induced a phosphorylation pattern more restricted than that achieved by LPS. Altogether these data indicate that proliferation signals induced by LPS or by the cross-linking of membrane immunoglobulins are controlled by late tyrosine phosphorylations occurring throughout the first 3 days of culture, controlled in part by protein kinase C activation, and dependent on the synthesis of an intermediate protein(s) either not secreted in the culture supernatant or present but biologically inactive in naive B cells.**

Resting murine B lymphocytes activated by lipopolysaccharide (LPS) proliferate and differentiate into antibody-secreting cells, whereas anti-membrane immunoglobulin M (IgM) antibodies (anti-μ Ab) induce only B-cell proliferation. The pattern of biochemical events induced by soluble anti-μ Ab has been well characterized. It involves activation of B-cell-receptor-associated protein tyrosine kinases (PTK) (9, 18), phosphorylation of phospholipases C (11), stimulation of phosphatidylinositol turnover (3), subsequent increase in intracellular $Ca^{2+}$, and activation of protein kinase C (PKC) (10). Early activation of PTK in anti-μ-activated B cells results in a typical pattern of tyrosyl phosphorylation (for reviews, see references 8 and 28). Conversely, the activation of B cells by LPS (3, 19), by multivalent agents (such as anti-Ig–dextran complexes) at low mitogenic concentrations (5), or by other T-cell-independent antigens with organized repeating epitopes (such as influenza virus) (36) is characterized by the absence of both detectable phosphatidylinositol turnover and $Ca^{2+}$ mobilization. It has been postulated that LPS could directly activate PKC (10) by mimicking diacylglycerol (4, 39). However, several facts argue against a unique role for PKC in LPS-induced B-cell activation. Firstly, direct activation of PKC by various phorbol esters does not promote B-cell proliferation but selectively induces differentiation into IgA-secreting plasma cells (31, 32) while down-regulating LPS-induced IgM and IgG expression (21). In contrast, the association of phorbol esters and calcium ionophores stimulates B-cell proliferation but does not induce differentiation into Ig-secreting cells (29). Secondly, cells depleted of PKC by prolonged treatment with phorbol esters fail to respond to anti-μ Ab but still respond to LPS (27).

While the activation of PTK in human monocytes (16, 33) and murine macrophages (38) stimulated with LPS has been amply demonstrated, Campbell and Sefton (9) and Brunswick et al. (6) reported the absence of tyrosine phosphorylations in the early steps of B-cell activation by LPS. In an apparent contradiction of these immunoblotting studies, Dearden-Badet and Revillard (13) reported that murine B-lymphocyte proliferation in response to LPS could be inhibited by the PTK inhibitors herbimycin A and genistein. Previous studies on signal transduction were performed within minutes following exposure to the activators. However, optimal B-cell proliferation cannot be achieved unless LPS (25) or anti-μ Ab (14) is present for several days. We therefore postulated that delayed signal transduction events could control cell proliferation. Here we report tyrosine phosphorylations occurring after several hours or days of stimulation by LPS and the mechanisms involved in the late signaling events.

## MATERIALS AND METHODS

**Mice.** Male BALB/c mice, 2 to 3 months old, were bred in our laboratory or purchased from IFFA Credo (L'Arbresle, France).

**Reagents.** LPS from *Salmonella typhimurium* (wild type) and phorbol 12-myristate 13-acetate (PMA) were from Sigma (St. Quentin Fallavier, France). Goat $F(ab')_2$ fragments specific for mouse IgM (anti-μ) were from Cappel (Durham, N.C.), and ionomycin was from Calbiochem (La Jolla, Calif.). Genistein, polymyxin B, herbimycin A, and chelerythrine were from Sigma.

**B-cell isolation and culture conditions.** Resting B cells were prepared from spleen by negative selection as previously described (31), with some modifications. Briefly, lymphocytes were separated from spleen cells by spinning over Lympholyte-M to eliminate macrophages. The isolated suspension was treated

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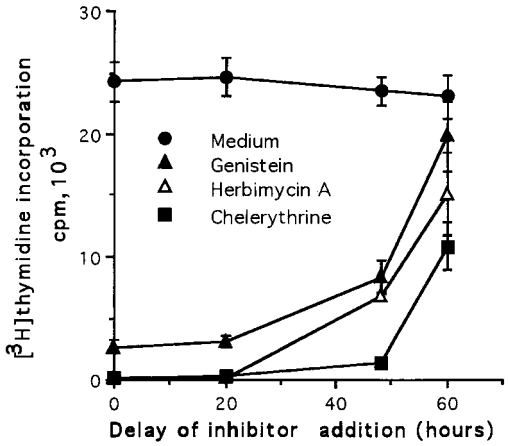


FIG. 1. The proliferative response to LPS is dependent on late activation of tyrosine kinases and serine/threonine kinase. Resting B cells were cultured for 72 h with 10 μg of *S. typhimurium* LPS/ml alone or with 50 μM genistein, 0.5 μM herbimycin A, or 2 μM chelerythrine added to wells 1 h before the indicated time, with time zero corresponding to the addition of LPS. The proliferative response was measured by [$^3$H]thymidine incorporation during the last 20 h of culture as indicated in Materials and Methods. Values are means ± standard errors of the means of three separate experiments.

with a mixture of monoclonal Ab to T lymphocytes (anti-Thy-1.2, HO 13.4; anti-L3T4, GK1.5; and anti-Ly-2, AD4) in the presence of rabbit complement (Cedarlane, Hornby, Canada). After a washing to eliminate dead cells, a suspension of enriched B cells ($2 \times 10^6$ cells/ml, 70 to 80% murine Ig-positive cells) was made in RPMI 1640 medium (Sigma) supplemented with 2 mM L-glutamine, $5 \times 10^{-5}$ M β-mercaptoethanol, 10% fetal calf serum, and an antibiotic-antimycotic mixture (100 U of penicillin/ml, 100 μg of streptomycin/ml, 250 ng of amphotericin B/ml) (Sigma).

**Proliferative responses.** B cells ($2 \times 10^5$ per well) were cultured in duplicate with the mitogens or medium in 96-well flat-bottom microtiter plates (Costar, Cambridge, Mass.) for various periods. One microcurie of [$^3$H]thymidine (Amersham, Little Chalfont, Buckinghamshire, United Kingdom) was added per well for the last 20 h of culture. Cells were harvested, and [$^3$H]thymidine incorporation was measured by liquid scintillation with a Packard scintillation counter.

**Preparation of cell lysates.** B cells ($3 \times 10^6$) were cultured, unless otherwise indicated, for 72 h in 24-well microtiter plates (Costar). Cells were harvested and then washed in ice-cold phosphate-buffered saline (PBS) supplemented with 100 mM sodium orthovanadate, and viable cells were counted by trypan blue exclusion. The pellet was solubilized with the volume of lysis buffer adjusted to $20 \times 10^6$ viable cells per ml. The lysis buffer contained the following: 2 mM EDTA, 10% glycerol, 1% Triton X-100, 133 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 21 μg of aprotinin/ml, and 10 μg of leupeptin/ml in 20 mM Tris-HCl buffer (pH 8). Lysis was carried out for 15 min on ice, and lysates were centrifuged for 15 min at 12,000 × *g* at 4°C, boiled with 2× Laemmli sample buffer, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% polyacrylamide).

**Western blotting.** After electrophoresis, proteins were transferred to a 0.45-μm-pore-size nitrocellulose sheet and incubated overnight at 4°C in deionized water with 3% $CH_3COOH$. The blots were blocked in PBS–0.1% Tween 20–4% bovine serum albumin. Tyrosine-phosphorylated proteins were revealed by incubating them with the 4G10 antiphosphotyrosine monoclonal Ab diluted 1:2,000 (Upstate Biotechnology Inc., Lake Placid, N.Y.) in blocking buffer for 1 h. Immunoreactive bands were visualized by incubating the blots for 45 min with goat anti-mouse IgG (heavy plus light chains) peroxidase F(ab′)$_2$ fragments (Bio-Rad, Richmond, Calif.), diluted 1:3,000 in PBS–0.1% Tween 20–5% milk, washed in PBS plus 0.1% Tween 20, and treated with the ECL kit (Amersham).

## RESULTS

**Late addition of PTK or PKC inhibitors prevents a B-cell proliferative response to LPS.** In order to assess the kinetics of PTK involvement in the B-cell mitogenic activity of LPS, two PTK inhibitors, genistein and herbimycin A, were added to the wells at different time intervals after stimulation by LPS, and [$^3$H]thymidine incorporation was measured. Genistein inhibits the cellular tyrosine kinases by competition for their ATP-binding site (1) whereas herbimycin A is a noncompetitive inhibitor which binds to the C-terminal domain of PTK and may induce their degradation (24). Both inhibitors completely inhibited LPS-induced proliferation when added before or up to 36 h after LPS, and they still decreased the response when added 48 h after LPS (Fig. 1). Furthermore, the PKC inhibitor chelerythrine (20) abrogated the proliferative response when added up to 48 h after LPS (Fig. 1).

**LPS-induced tyrosine phosphorylations are detectable after 48 h of culture.** To determine whether a PTK-specific pathway was involved during LPS activation, tyrosine-phosphorylated proteins were assessed by Western blotting of lysates from murine B cells stimulated with *S. typhimurium* wild-type LPS (10 μg/ml) for 24, 48, or 72 h. Knowing that cell proliferation and cell death during culture differ according to experimental conditions, the lysis buffer volume in each sample was adjusted to the viable cell number determined by trypan blue exclusion. Therefore, phosphotyrosine patterns in each lane are derived from the same number of viable cells. In unstimulated cultures, recovery of viable cells dropped to 50% of seeded B cells after 24 h, so cell lysates obtained beyond this time, which showed little or no tyrosyl phosphorylation, could not be used for reliable analysis. Results were compared with early tyrosine phosphorylations in B cells activated with anti-μ for 1 to 60 min. The maximal response obtained after 10 min is presented in Fig. 2A. Anti-μ induced rapid tyrosine phosphorylations of proteins with relative molecular masses of 104, 100, 47, 43, and 36 kDa as well as an increase in intensity of proteins that

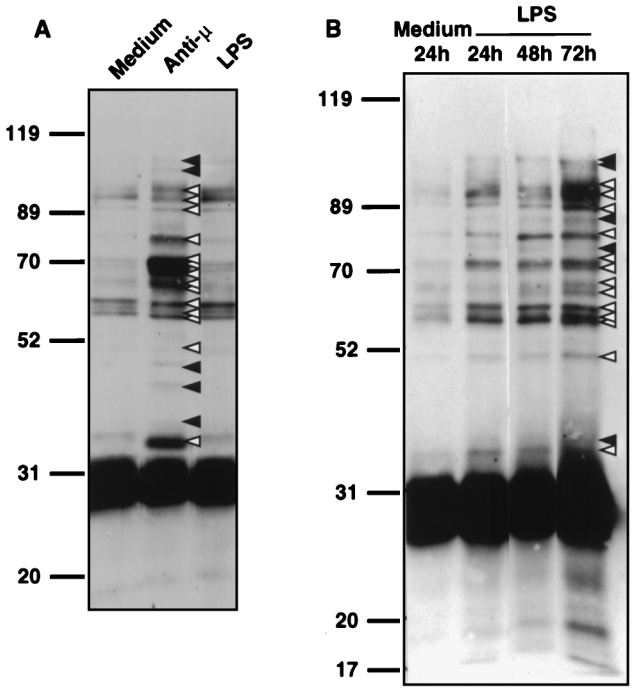


FIG. 2. Tyrosine phosphorylations induced by LPS increase as a function of culture duration. Resting B cells were cultured with 10 μg of anti-μ/ml, 10 μg of *S. typhimurium* LPS/ml, or medium only for 10 min (A) or with 10 μg of *S. typhimurium* LPS/ml or medium only for the indicated times (B). Proteins were extracted from the same number of viable cells and analyzed for phosphotyrosines by immunoblotting as indicated in Materials and Methods. The proliferation indices after 24, 48, and 72 h of activation with LPS were 4, 13, and 20, respectively. Filled arrowheads indicate activation-induced tyrosine-phosphorylated proteins at 104, 100, 47, 43, and 36 kDa (A) and 103, 99, 82, 74, and 37 kDa (B). Empty arrowheads indicate activation-dependent intensity increase of spontaneously tyrosine-phosphorylated proteins at 94, 90, 87, 77, 70, 69, 65, 63, 60, 57, 56, 50, and 34 kDa (A) and 93, 89, 86, 78, 71, 70, 65, 64, 61, 58, 57, 49, and 35 kDa (B).

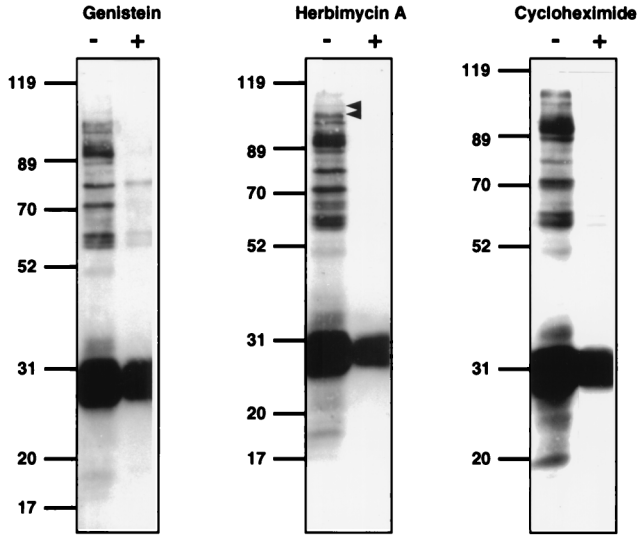


FIG. 3. LPS-induced tyrosine phosphorylations are inhibited by the PTK inhibitors genistein and herbimycin A and are dependent on protein synthesis. Resting B cells were incubated for 72 h with 10 μg *S. typhimurium* LPS/ml alone (−) or in the presence (+) of 50 μM genistein, 0.5 μM herbimycin A, or a 0.5 μM concentration of the protein synthesis inhibitor cycloheximide as indicated. Inhibitors were added 1 h before LPS. Tyrosine phosphorylations in cell lysates were analyzed as described in Materials and Methods. The arrowheads indicate the 103- and 109-kDa phosphoproteins.

tyrosyl phosphorylated spontaneously in unactivated cells. As expected, we failed to detect any LPS-induced tyrosine phosphorylation distinct from that observed in inactivated cells during the first hour of activation (Fig. 2A). However, LPS induced phosphorylations of tyrosines observed several hours after the beginning of the culture.

As shown in Fig. 2B, four major LPS-induced tyrosine-phosphorylated proteins of 103, 99, 82, and 74 kDa were observed after 24 h, and their intensities increased up to 72 h of culture; two other specifically induced phosphoproteins of 109 and 37 kDa were also seen (these are better demonstrated in Fig. 3 and 5). Other tyrosine-phosphorylated proteins were observed in unstimulated cells after 24 h of culture, but their intensities increased as a function of time in the presence of LPS. Their molecular masses (93, 89, 86, 78, 71, 70, 65, 64, 61, 58, 57, 49, and 35 kDa) were quite similar to those increased by anti-μ earlier (Fig. 2A and B). Therefore, the kinetics of increase in tyrosine-phosphorylated proteins follows that of [$^3$H]thymidine incorporation and reaches a maximum after 72 h of culture with LPS (reference 25 and proliferation indices in the legend to Fig. 2). Total inhibition of tyrosine phosphorylations was observed with both inhibitors of tyrosine kinases, genistein and herbimycin A (Fig. 3).

**LPS-induced tyrosine phosphorylation requires de novo protein synthesis.** The delayed appearance of tyrosine-phosphorylated proteins following activation by LPS might be attributed to PTK activation by cytokines released from B cells, as suggested by previous reports (7, 12). To address this hypothesis we assessed tyrosine phosphorylation profiles of LPS-activated B cells cultured in the presence of cycloheximide. As shown in Fig. 3, tyrosine phosphorylations were completely inhibited. Furthermore, supernatants of B cells cultured for 24, 48, or 72 h with LPS were tested for induction of tyrosine phosphorylations on freshly prepared B cells after 24 h of exposure. LPS present in the supernatants was neutralized with the cationic antibiotic polymyxin B. As previously shown, LPS alone induced little tyrosine phosphorylation after 24 h, while supernatants, with or without polymyxin B, enhanced phosphorylation. However, none of the supernatants tested reproduced the pattern observed in cells activated for 72 h with LPS (Fig. 4A). Supernatants failed to induce tyrosine phosphorylations in the minutes following addition of LPS to cells (data not shown). Furthermore, the B-cell proliferative response to su-

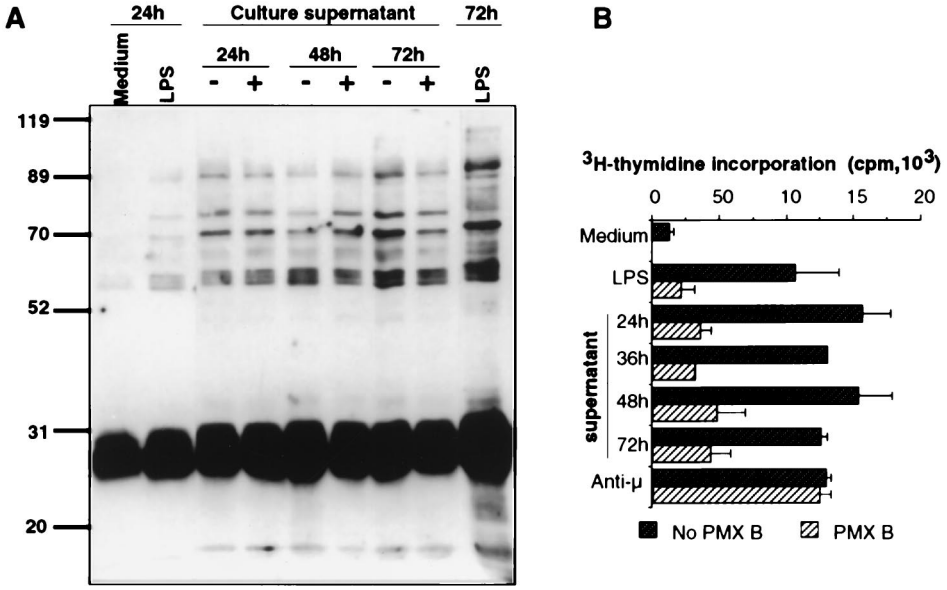


FIG. 4. LPS-mediated tyrosine phosphorylations do not result from cytokine release. Supernatants of B cells activated with 10 μg of *S. typhimurium* LPS/ml for the indicated times were added to freshly prepared B cells alone (−) or in the presence of polymyxin B (50 μg/ml) (+). (A) Tyrosine phosphorylations in cell lysates were analyzed after 24 h of culture as described in Materials and Methods. Tyrosine-phosphorylated proteins in cells cultured for 24 h with medium alone or with LPS or for 72 h with LPS are represented. (B) The B-cell proliferative response was measured after 48 h of culture with the supernatants, medium alone, LPS from *S. typhimurium* (10 μg/ml), or anti-μ Ab (10 μg/ml), with or without polymyxin B (PMX B) (50 μg/ml).

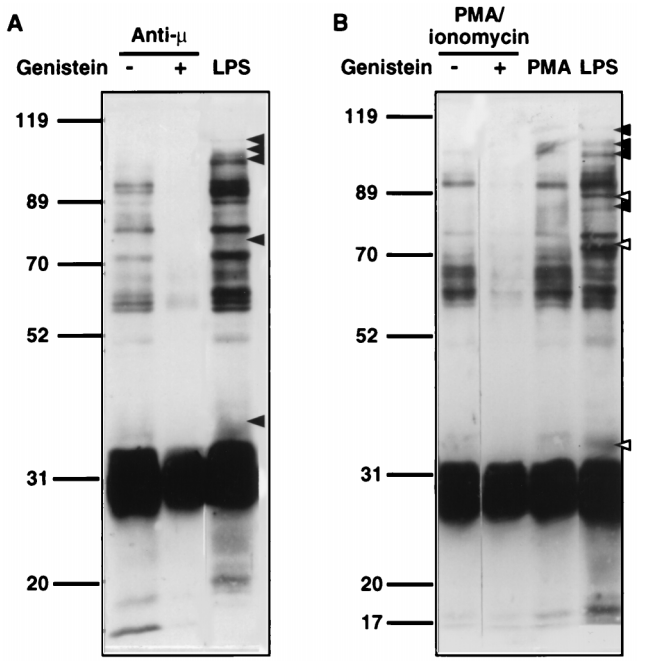


FIG. 5. Late tyrosine phosphorylations in B cells stimulated by different activators. Resting B cells were incubated for 72 h with LPS alone (10 μg/ml) or with anti-μ Ab (10 μg/ml) with (+) or without (−) genistein (A) or with PMA plus ionomycin (10 ng/ml and 1 μg/ml, respectively) with (+) or without (−) genistein or PMA only (10 ng/ml) (B). Cells were lysed and tyrosine-phosphorylated proteins were analyzed as described in Materials and Methods. In each panel, arrowheads indicate the phosphorylated proteins that are present only in LPS-stimulated cells with apparent molecular masses of 109, 103, 99, 74, and 37 kDa (A) and 109, 103, 99, 86, 82, 71, and 35 kDa (B). Filled arrowheads indicate activation-induced tyrosine-phosphorylated proteins, and empty arrowheads indicate activation-dependent increases in intensity of spontaneously tyrosine-phosphorylated proteins.

pernatants was inhibited by polymyxin B, indicating that no mitogenic activity could be achieved in the absence of LPS (Fig. 4B). Polymyxin B inhibition was not due to toxicity since the response to anti-μ Ab was not altered. Altogether, these results indicate that PTK activation triggered by LPS does not depend solely on soluble mediators released in culture supernatants but involves late events which require protein synthesis and the continuous presence of LPS.

**Other B-cell mitogens induce delayed PTK activation.** To determine whether late PTK activation was specific for LPS or common to other B-cell mitogens, antiphosphotyrosine Western blotting was performed on cells incubated for 3 days with anti-μ Ab or with a combination of PMA and calcium ionophores. As shown in Fig. 5, the pattern of tyrosine-phosphorylated proteins induced by anti-μ Ab was quite similar to that observed with LPS, with a few exceptions: anti-μ Ab did not induce the phosphorylation of the 109-, 99-, and 74-kDa proteins, whereas the 103- and 37-kDa proteins were not detectable (only a 35-kDa protein was present) in four of five separate experiments. The tyrosyl-phosphorylated proteins were observed in B cells incubated for 72 h with a mixture of PMA and ionomycin, albeit with a reduced number of phosphorylated substrates. The phosphorylation of the 109-, 99-, and 82-kDa proteins was not induced, the phosphorylation of the 103- and 71-kDa proteins was not always detectable, and spontaneous phosphorylation of some proteins (e.g., those of 86 and 35 kDa) was not maintained. In contrast, activation with PMA restored a more complete pattern of tyrsoyl phosphorylations than occurred in the presence of ionomycin, with detectable tyrosyl phosphorylations of the 103-, 99-, 82-, and 71-kDa proteins (Fig. 5B and 6).

**PKC dependence of tyrosine phosphorylation.** To assess the role of PKC in PTK activation, tyrosine phosphorylations were analyzed with cells stimulated with PMA only. As shown in Fig. 5, the same pattern was observed in PMA- and PMA-ionomycin-stimulated cells. All tyrosine phosphorylations were inhibited in the presence of genistein (Fig. 5) or herbimycin A (data not shown). To get further evidence for the role of PKC, cells were treated with the PKC inhibitor chelerythrine before addition of the activators. As shown in Fig. 6, chelerythrine inhibited LPS-induced, anti-μ Ab-induced, and, with a stronger effect, PMA-induced late tyrosine phosphorylations.

## DISCUSSION

The aim of this study was to assess the role of PTK in LPS-induced activation of mouse B cells, with special emphasis on the late signals which control B-cell proliferation. The results presented here demonstrate that B-cell activation triggered by LPS involves not only a PKC-dependent but also a PTK-dependent pathway.

Previous studies established that PTK activation by anti-μ Ab occurred within minutes following stimulation and persisted after 1 h (Fig. 2A) (9, 18), whereas no tyrosyl phosphorylation was observed in the presence of LPS (Fig. 2A) or phorbol ester plus calcium ionophores within the same period (5, 30). However, kinetic studies indicate that in the presence of either anti-μ Ab or LPS, PTK activation occurs during the first day and increases over time until day 3, while tyrosyl phosphorylation in unstimulated cells is no longer detectable after 24 h.

The presence of phosphotyrosyl proteins might be attributed at least in part to the inhibition of tyrosine phosphatases because some of the proteins that spontaneously tyrosyl phosphorylated in unstimulated cultures until 24 h were maintained in mitogen-stimulated cells until 72 h. However, late tyrosyl

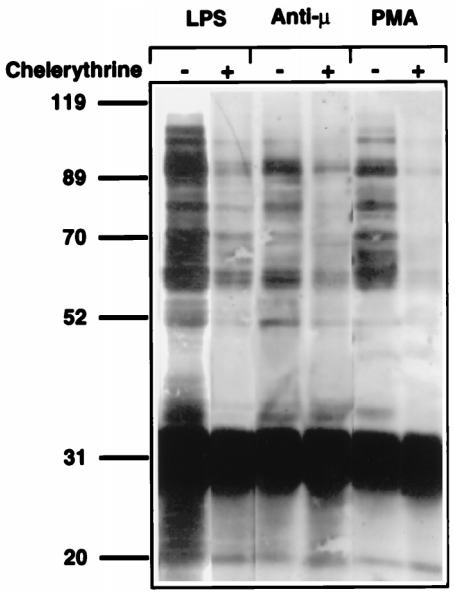


FIG. 6. Late tyrosine kinase activation is dependent on PKC activation. Resting B cells were stimulated for 72 h with LPS (10 μg/ml), anti-μ Ab (10 μg/ml), or PMA (10 ng/ml) alone (−) or in the presence of chelerythrine (2 μM) added 1 h before the activators (+). Cells were lysed and tyrosine-phosphorylated proteins were analyzed as described in Materials and Methods.

phosphorylations were dependent primarily on PTK activation because (i) several substrates were specific for activated cells and (ii) protein phosphorylation was inhibited by genistein and herbimycin A.

The patterns of late tyrosyl phosphorylations demonstrated some similarities between LPS- and anti-μ-stimulated B cells, but some substrates appeared to be specific for LPS activation (e.g., proteins of 109 and 74 kDa). Quite similar phosphotyrosyl patterns were observed with B cells activated by LPS from *Escherichia coli* O111:B4 and *Salmonella minnesota* Re595 (data not shown).

Some similarities between the late tyrosyl phosphorylations induced by LPS and those initially induced by anti-μ were observed. For instance, proteins of 104, 100, and 36 kDa phosphorylated after 10 min of anti-μ stimulation may be similar to the 103-, 99-, and 37-kDa phosphoproteins observed with LPS (Fig. 2). On the other hand, some substrates (e.g., proteins of 47 and 43 kDa) were observed in the minutes following anti-μ addition and disappeared thereafter. Of note, a phosphoprotein of approximately 82 kDa was observed after a long exposure to either LPS or anti-μ (Fig. 5).

To bypass the increase in cell number during B-cell activation, tyrosine phosphorylations were measured on the same number of viable cells. Although the increase in volume of B cells within 24 h of activation may account for the increase in intensity of tyrosine phosphorylations in activated compared to unactivated cells, it can account for neither the increase in tyrosyl phosphorylation intensity between 24 and 72 h nor the tyrosyl phosphorylation of new substrates. Furthermore, the increase in intensity during this period was not observed on all substrates (e.g., the doublet of 56 and 60 kDa).

Delayed kinetics and the similarities between both patterns of tyrosine phosphorylations suggest a role for PTK inducers common to LPS and anti-μ Ab as, for instance, cytokines secreted by activated B cells. Supporting this hypothesis, LPS-induced tyrosine phosphorylations were dependent on de novo protein synthesis. A similar inhibition by cycloheximide was observed in anti-μ-induced tyrosine phosphorylations (data not shown). The possible role of soluble mediators was addressed by assessing the activity of LPS-stimulated B-cell supernatants on fresh B cells. Such supernatants were devoid of mitogenic activity when LPS was neutralized by polymyxin B. Conversely, the same supernatants were shown to induce tyrosine phosphorylations in fresh B cells and LPS did not contribute to this effect. However, the phosphorylation pattern induced by supernatants was restricted to only some of the substrates that become phosphorylated after 72 h of exposure to LPS. Therefore, the data indicate that the presence of LPS is required to achieve the complete pattern of PTK activation but that soluble mediators released by activated B cells may contribute to PTK activation.

In contrast to early tyrosine phosphorylations induced by anti-μ Ab that occur prior to PKC activation (5, 23), several lines of evidence support a role for PKC in late tyrosyl phosphorylations. Firstly, direct activation of PKC by PMA induced tyrosyl phosphorylations of some substrates which were also observed after 72 h of activation with LPS or anti-μ Ab. Secondly, late tyrosyl phosphorylations induced by LPS or anti-μ Ab were completely suppressed by the PKC inhibitor chelerythrine. Altogether, these data suggest that PKC activation is essential for late PTK activation but that additional pathways are probably also involved, as suggested by the restricted spectrum of tyrosine phosphorylations induced by phorbol ester alone compared with those induced by anti-μ Ab or LPS. PMA alone fails to induce proliferation, probably because of the PKC depletion following a sustained translocation to the membrane (34, 35), while addition of calcium ionophores restores the proliferative response (29). However, the pattern of tyrosine phosphorylations observed in cells cultivated with PMA plus ionomycin was obtained with PMA only, indicating that $Ca^{2+}$ mobilization was not required for PTK activation.

Direct activation of PKC by phorbol esters has previously been reported to increase PTK activity in other cell types (17, 22) and to modulate the activity of membrane (15) and receptor-associated (37) tyrosine kinases that are involved in the modulation of antigen (2) or epidermal growth factor receptors (37). Late tyrosine phosphorylations may result from interaction of anti-μ Ab, LPS, or induced cytokines with newly expressed receptors dependent on PKC activation and protein synthesis. Recent results from our laboratory show that mitogenic stimulation of murine B cells results in a progressive and sustained increase of LPS binding sites over 3 days, suggesting that late PTK activation could result from interaction between LPS and these newly expressed receptors.

The results presented here are in keeping with a recent report by Karras et al. (26) showing delayed tyrosine phosphorylation and activation of the STAT1 transcription factor in B lymphocytes activated by anti-μ Ab or PMA plus ionomycin. STAT1 activation was dependent on PKC activation and protein synthesis but did not involve a cytokine-like pathway. Altogether, the present results lead us to conclude that early signaling events occurring within minutes of mitogen addition are not representative of the whole activation process of B lymphocytes. A more complex sequence of events involving late PTK activation dependent on (i) de novo protein synthesis, (ii) PKC activation, and (iii) the presence of LPS is required for the mitogenic response to proceed. Further studies are obviously needed to get a better insight into the late signaling cascade, to identify the tyrosyl-phosphorylated targets, and to ascertain the precise relationship between PTK activation and the mitogenic activity of LPS. However, the complete abrogation of B-cell proliferation by PTK and PKC inhibitors added up to 48 h after LPS provides strong indirect evidence for a major role of late signaling events in B-cell mitogenic responses.

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